

Figure 1 (A-F)

Construct Forms Comprising at Least one Single-Stranded Region



2/23

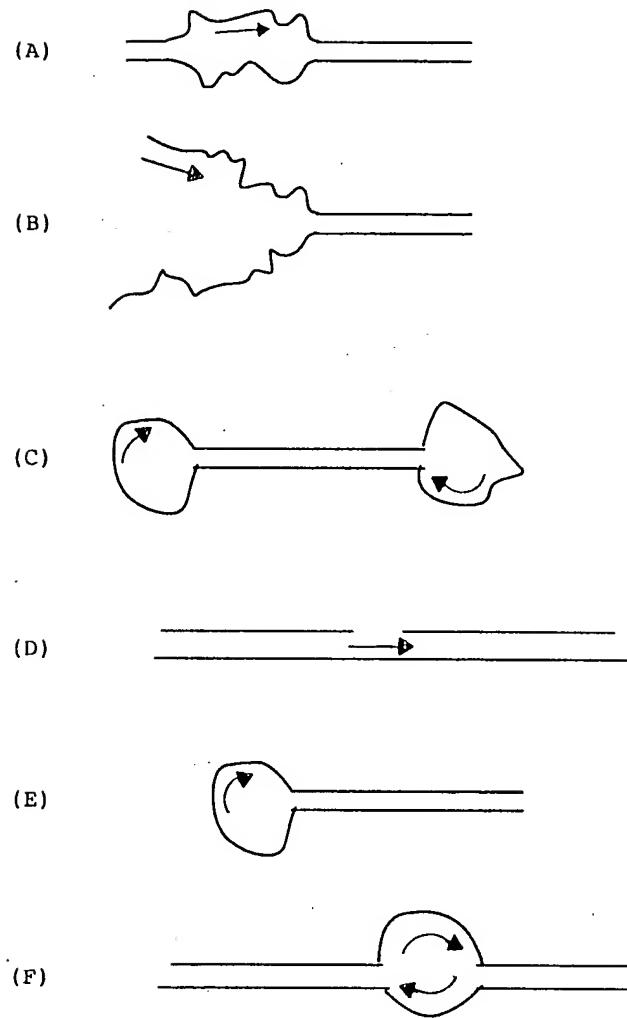


Figure 2 (A-F)

Functional Forms of the Construct



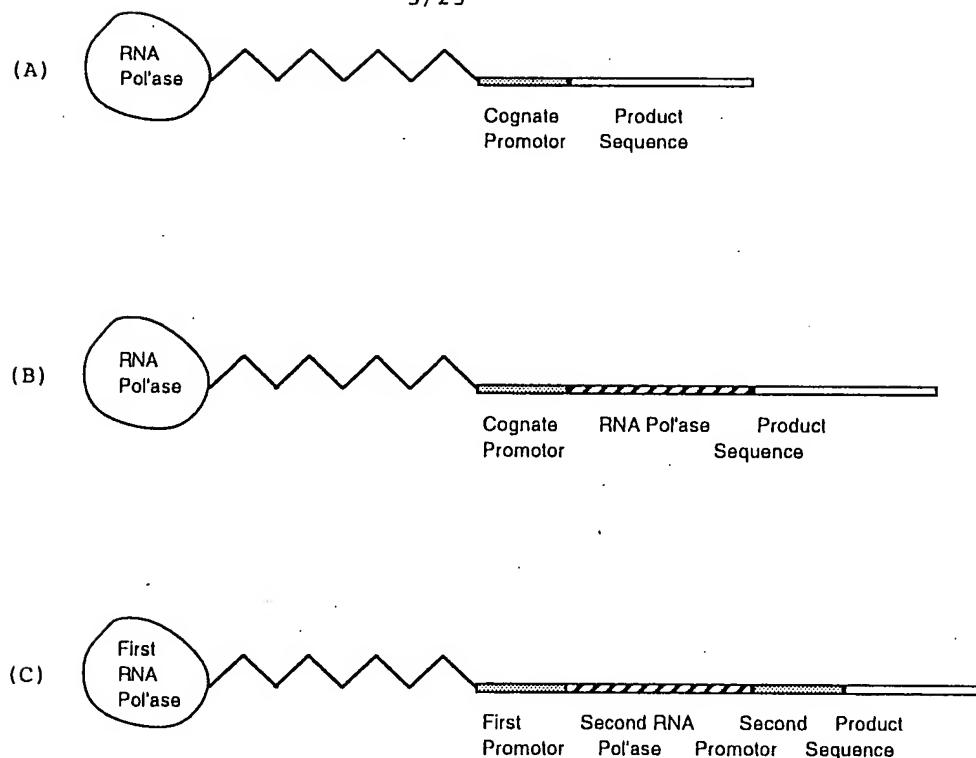


Figure 3 (A-C)

Three Constructs with an RNA Polymerase
Covalently Attached to a Transcribing Cassette

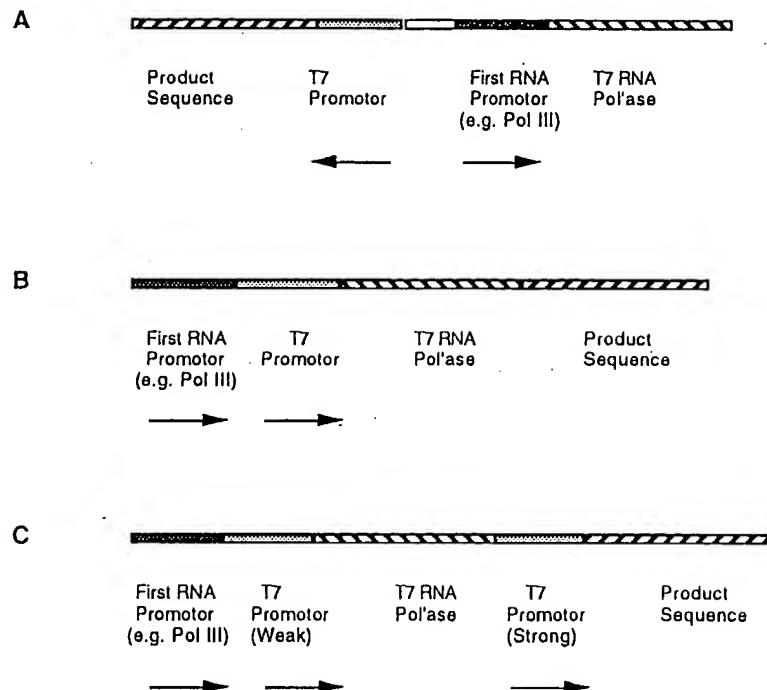


Figure 4 (A-C)

**Three Constructs with Promoters
for Endogenous RNA Polymerase**

M13mp18. Seq Length: 7250

1. AATGCTACTA CTATTAGTAG AATTGATGCC ACCTTTTCAG CTOGOGOCOC
 51. AAATGAAAAT ATAGCTAAC AGGTTATTGA CCATTTGCGA AATGTATCTA
 101. ATGGTCAAAC TAAATCTACT CGTTTGCAGA ATTGGGAATC AACTGTTACA
 151. TGGAATGAAA CTTOCAGACA CGTACTTTA GTTGCATATT TAAAACATGT
 201. TGAGCTACAG CACCAGATTG AGCAATTAAG CTCTAAGCCA TCGCAAAAA
 251. TGACCTCTTA TCAAAAGGAG CAATTAAGG TACTCTCTAA TCGTACCTG
 301. TTGGAGTTTG CTTOCGGTCT GGTTGCTTT GAAGCTGAA TAAAAAOOG
 351. ATATTTGAAG TCTTTOGGGC TTCTCTTAA TCTTTTGAT GCAATCGCT
 401. TTGCTTCTGA CTATAATAGT CAGGGTAAAG ACCTGATTT TGATTTATGG
 451. TCATTCTCGT TTTCTGAAC GTTTAAAGCA TTTGAGGGGG ATTCAATGAA
 501. TATTTATGAC GATTOCGAG TATGGACGC TATCCAGTCT AAACATTTA
 551. CTATTACCCCC CTCTGGCAAA ACTTCTTTG CAAAGCCTC TCGCTATTT
 601. GGTTTTATC GTGCGTCTGGT AAAAGGGGT TATGATAGTG TTGCTCTTAC
 651. TATGCGCTGT AATTCTTTT GGGCGTTATGT ATCTGCATTA GTTGAATGTG
 701. GTATTCCTAA ATCTCAACTG ATGAATCTT CTACCTGTAA TAATGTTGTT
 751. CGTTAGITC GTTTTATTAA CGTAGATTT TCTTCCCAAC GTCTGACTG
 801. GTATAATGAG CCAGTTCTTA AAATCGCATA AGGTAATTCA CAATGATTAA
 851. AGTTGAAATT AAACCATCTC AAGGCCAATT TACTACTCGT TCTGGTGTTC
 901. TCGTCAGGGC AAGCTTATT CACTGAATGA GCAGCTTGT TACGTTGATT
 951. TGGGTAATGA ATATCGGTT CTGTCGAAG ATTACTCTG ATGAAGGTCA
 1001 GCGAGCTAT GCGCGTGGTC TGTCACCGT TCATCTGTOC TCTTCAAG
 1051 TTGGTCAGTT OGGTTCCCTT ATGATTGACC GTCTGCGCT CGTTGGGCT
 1101 AAGTAACATG GAGCAGGTOG OGGATTGGA CACAATTAT CAGGGCGATGA
 1151 TACAAATCTC CGTTGTACCTT TGTTTGGCG TTGGTATAAT CGCTGGGGGT
 1201 CAAAGATGAG TGTTTAGTG TATTCTTCTG CCTCTTGT TTTAGGTTGG

Figure 5

M13mp18 Nucleic Acid Sequence

1251	TGCTTGT	GTGGCATTAC	GTATTTACC	CGTTAATGG	AAACTTCCTC
1301	ATGAAAAAGT	CTTTAGTCCT	CAAAGCTCT	GTAGGCGTTG	CTACOCTG
1351	TOCGATGCTG	TCTTTCGCTG	CTGAGGGTGA	CGATCOOGCA	AAAGOGGCGT
1401	TTAACTCCCT	GCAAGOCTCA	GOGACOOGAAT	ATATOGGTTA	TGCGTGGGCG
1451	ATGGGTTGTTG	TCATTGTCGG	CGCAACTATC	GGTATCAAGC	TGTTTAAGAA
1501	ATTCAACCTCG	AAAGCAAGCT	GATAAACOGA	TACAATTAAA	GGCTOCTTTT
1551	GGAGCCTTTT	TTTTGGAGA	TTTCAACGT	GAAAAAAATTA	TTATTCGCAA
1601	TTCCTTTAGT	TGTTTCTTTC	TATTCTCACT	CGCTGAAAC	TGTTGAAAGT
1651	TGTTTAGCAA	AAACCCATAC	AGAAAATTCA	TTTACTAACG	TCTGAAAGA
1701	CGACAAAACT	TTAGATCGTT	ACGCTAACTA	TGAGGGTTGT	CTGTGGAATG
1751	CTACAGGCGT	TGTAGTTTGT	ACTGGTGAOG	AAACTCAGTG	TTACGGTACA
1801	TGGGTTCTA	TTGGGCTTGC	TATCCTGAA	AATGAGGGTG	GTGGCTCTGA
1851	GGGTGGCGGT	TCTGAGGGTG	CGGGTTCTGA	GGGTGGCGGT	ACTAAACCTC
1901	CTGAGTAogg	TGATACACCT	ATTCGGGCT	ATACTTATAT	CAACCCCTCTC
1951	GACGGCACTT	ATCGGCTGG	TACTGAGCAA	AAACCGCTA	ATCGTAATOC
2001	TTCTCTTGAG	GAGTCCTCAGC	CTCTTAATAC	TTTCATGTTT	CAGAATAATA
2051	GGTTOGAAA	TAGCCAGGGG	GCATTAACGT	TTTATAOGGC	CACTGTTACT
2101	CAAGGCACTG	ACCCCGTTAA	AACTTATTAC	CAGTACACTC	CTGTATCATC
2151	AAAAGCCATG	TATGACGCTT	ACTGGAAOGG	TAAATTCAGA	GAATGCGCTT
2201	CAAGGCACTG	ACCCCGTTAA	AACTTATTAC	CAGTACACTC	CTGTATCATC
2151	AAAAGCCATG	TGCTCAACC	TOCTGTCAAT	GCTGGCGGGCG	GCTCTGGTGG
2201	TOCATTCTGG	CTTTAATCAA	GATCCATTG	TTTGTGAATA	TCAAGGCCAA
2251	TOGTCGACC	TGCTCAACC	TOCTGTCAAT	GCTGGCGGGCG	GCTCTGGTGG
2301	TGGTTCTGGT	GGGGGCTCTG	AGGGTGGTGG	CTCTGAGGGT	GGGGGTTCTG
2351	AGGGTGGCGG	CTCTGAGGGG	GGGGGTTGG	GTGGTGGCTC	TGGTTGGGT
2401	GATTTGATT	ATGAAAAGAT	GGCAAACGCT	AATAAGGGGG	CTATGACCGA
2451	AAATGCGAT	AAAAACCGCG	TACAGTCTGA	CGCTAAAGGC	AAACTTGATT

Figure 5

M13mp18 Nucleic Acid Sequence

2501	CTGTOGCTAC	TGATTACGGT	GCTGCTATCG	ATGGTTTCAT	TGGTGACGTT
2551	TCGGGCTTIG	CTAATGGTAA	TGGTGCTACT	GGTGATTTG	CTGGCTCTAA
2601	TTCOCAAATG	GCTCAAGTCA	GTGACGGTGA	TAATTACACCT	TTAATGAATA
2651	ATTCGCGTCA	ATATTTACCT	TOOCTCCCTC	AATGGTTGA	ATGTCGOCCT
2701	TTTGTCTTAA	GGCGCTGGTAA	ACCATATGAA	TTTTCTATTG	ATTGTGACAA
2751	AATAAACTTA	TTCGGTGGTG	TCTTGCGTT	TCTTTTATAT	GTTGCGACCT
2801	TTATGTATGT	ATTTTCTACG	TTTGCTAACCA	TACTGCGTAA	TAAGGAGCT
2851	TTATCATGCC	AGTTCTTTG	GGTATTCGGT	TATTATTGCG	TTTCTCGGT
2901	TTCCTCTGG	TAACCTTGTG	GGGCTATCTG	CTTACTTTTC	TTAAAAAGGG
2951	CTTOGTAAG	ATAGCTATTG	CTATTCATT	GTTCCTTGCT	CTTATTATTG
3001	GGCTTAACTC	AATTCTTGTG	GGTTATCTCT	CTGATATTAG	CGCTCAATT
3051	CCCTCTGACT	TTGTCAGGG	TGTTCAAGTTA	ATTCTCCCGT	CTAATGCGCT
3101	TCCCTGTTTT	TATGTTATTTC	TCTCTGTAAA	GGCTGCTATT	TTCATTTTG
3151	ACGTTAAACA	AAAAATCGTT	TCTTATTGG	ATTGGGATAA	ATAATATGGC
3201	TGTTTATTTC	GTAACCTGGCA	AATTAGGCTC	TGGAAAGACG	CTCGTTAGCG
3251	TTGGTAAGAT	TCAGGATAAA	ATTGTAGCTG	GGTGCAAAAT	AGCAACTAAT
3301	CTTGATTTAA	GGCTTCAAAA	OCTCCCGCAA	GTOGGGAGGT	TCGCTAAAAC
3351	GGCTCGCGTT	CTTAGAATAC	CGGATAAGOC	TTCTATATCT	GATTTGCTTG
3401	CTATTGGCG	CGGTAATGAT	TOCTACGAATG	AAAATAAAA	GGCTTGCTT
3451	GTTCTCGATG	AGTGCGGTAC	TTGGTTTAAT	ACCGTTCTT	GGAATGATAA
3501	GGAAAGACAG	CGGATTATTG	ATTGGTTCT	ACTGCTGTT	AAATTAGGAT
3551	GGGATATTAT	TTTCTTGTGTT	CAGGACTTAT	CTATTGTTGA	TAACAGGCG
3601	CGTTCTGCAT	TAGCTGAACA	TGTTGTTAT	TGTCGTOGTC	TGGACAGAAT
3651	TACTTACCT	TTTGTGGTA	CTTTATATT	TCTTATTACT	GGCTGAAAAA
3701	TGCTCTGOC	TAAATTACAT	GTTGGCGTTG	TTAAATATGG	CGATTCTCAA
3751	TTAAGCGCTA	CTGTTGAGCG	TTGGCTTTAT	ACTGGTAAGA	ATTTGTATAA
3801	CGCATATGAT	ACTAACACGG	CTTTTCTAG	TAATTATGAT	TCGGGTGTTT

Figure 5

M13mp18-Nucleic Acid Sequence

3851	ATTCTTATTT	AAACGCTTAT	TTATCACACG	GTCGGTATTT	CAAACATTA
3901	AATTAGGTC	AGAAGATGAA	ATTAACAAA	ATAATATTGA	AAAAGTTTC
3951	TCGCGTTCTT	TGTCTTGGGA	TTGGATTGTC	ATCAGCATT	ACATATAGTT
4001	ATATAACCCA	ACCTAACGCG	GAGGTAAAAA	AGGTAGTCCTC	TCAGACCTAT
4051	GATTTGATA	AATTCACTAT	TGACTCTTCT	CAGGGTCTTA	ATCTAAGCTA
4101	TCGCTATGTT	TTCAAGGATT	CTAAGGGAAA	ATTAATTAAT	AGOGACGATT
4151	TACAGAAGCA	AGGTTATTCA	CTCACATATA	TTGATTATG	TACTGTTCC
4201	ATAAAAAAAG	GTAATTCAAA	TGAAATTGTT	AAATGTAATT	AATTTGTTT
4251	TCTTGATGTT	TGTTTCATCA	TCTTCCTTGT	CTCAGGTAAT	TGAAATGAAT
4301	AATTGCGTC	TGCGCGATT	TGTAACCTGG	TATTCAAAGC	AATCAGGCGA
4351	AATCCGTTATT	GTTTCTCGG	ATGTAAAAGG	TACTGTTACT	GTATATTCT
4401	CTGACGTTAA	ACCTGAAAAT	CTACCGCAATT	TCTTATTTTC	TGTTTAACT
4451	GCTAATAATT	TTGATAATGGT	TGGTTCAATT	CCTTCATAAA	TTCAGAAGTA
4501	TAATCCAAAC	AATCAGGATT	ATATTGATGA	ATTGCCATCA	TCTGATAATC
4551	AGGAATATGA	TGATAATTCC	GCTCTTCTG	GTGGTTCTT	TGTTTGGCAA
4601	AATGATAATG	TTACTCAAC	TTTAAATT	AATAACGTT	GGGAAAGGA
4651	TTAACATCGA	GTTGCGAAT	TGTTGTAAA	GTCTAATACT	TCTAAATCCT
4701	CAAATGTATT	ATCTATTGAC	GGCTCTAAC	TATTAGTTGT	TAGTGCTOCT
4751	AAAGATATT	TAGATAACCT	TCCTCAATT	CTTCTACTG	TTGATTGOC
4801	AACTGACCAAG	ATATTGATTG	AGGGTTGAT	ATTGAGGTT	CAGCAAGGTG
4851	ATGCTTCTAGA	TTTTTCATT	GCTGCTGGCT	CTCAGOGTGG	CACTGTTGCA
4901	GGGGTGTATA	ATACTGACCG	CCTCACTCT	GTTTATCTT	CTGCTGGTGG
4951	TTCGTTGGT	ATTTTAATG	GCGATGTTT	AGGGCTATCA	GTTOGOGCAT
5001	TAAAGACTAA	TAGOCATTCA	AAAATATTGT	CTGTGOCAG	TATTCTTACG
5051	CTTCAGGTC	AGAAGGGTTC	TATCTCTGTT	GGCAGAATG	TCCCTTTAT
5101	TAAAGACTAA	TAGOCATTCA	AAAATATTGT	CTGTGOCAG	TATTCTTACG
5151	CGATTGAGCG	TCAAAATGTA	GGTATTGCA	TGAGCGTTT	TCCTGTTGCA

Figure 5

M13mp18 Nucleic Acid Sequence

5201	ATGGCTGGCG	GTAATATTGT	TCTGGATATT	ACCAAGCAAGG	CGGATAGTTT
5251	GAGTTCTCT	ACTCAGGCAA	GTGATGTTAT	TACTAATCAA	AGAAGTATTG
5301	CTACAAACGGT	TAATTTGGGT	GATGGACAGA	CTCTTTACT	GGGTGGACTC
5351	ACTGATTATA	AAAACACTTC	TCAAGATTCT	GGGGTACCGT	TOCTGTCTAA
5401	AATCCCTTAA	ATGGCGCTCC	TGTTTAGCTC	CGCGCTCTGAT	TOCAACGAGG
5451	AAAGCACGTT	ATACGTGCTC	GTCAAAGCAA	CCATAGTAOG	CGCGCTGTAG
5501	GGGGCGATTAA	AGGGGGGGG	GTGTGGTGGT	TAOGCGCAGC	GTGACCGCTA
5551	CACTTGCGAG	CGCGCTAGCG	CGCGCTCTT	TCGCTTTCTT	CGCTTCCTT
5601	CTGGCGAAGT	TOGGGGCTT	TOCCCGTCAA	GCTCTAAATC	GGGGGGCTCC
5651	TTTACGGGTC	CGATTTAGTG	CTTTACGGCA	OCTGGACCC	AAAAAACTTG
5701	ATTTGGGTGA	TGGTTCACTG	AGTGGGCGAT	CGCGCTGATA	GAOGGTTTTT
5751	CGCCCTTTGA	CGTTGGAGTC	CAOGTTCTT	AATAGTGGAC	TCTTGTTCGA
5801	AACTGGAAACA	ACACTCAACC	CTATCTGGG	CTATTCTTT	GATTATAAG
5851	GGATTTGCC	GATTTGGAA	CCACATCAA	ACAGGATTT	CGCGCTGG
5901	GGCAAACCAAG	CGTGGACCGC	TTGCTGCAAC	TCTCTCAGGG	CGAGGGGGTG
5951	AAGGGCAATC	AGCTGTTGCC	CGCTCGCTG	GTGAAAAGAA	AAACCAACCT
6001	GGGGCGAACAT	AOGCAAAACG	OCTCTCOOOG	CGCGTTGGGC	GATTCACTAA
6051	TGCAAGCTGGC	AOGACAGGTT	TOCCGACTGG	AAAGCGGGCA	GTGAGGGCAA
6101	CGCAATTAAAT	GTGAGTTAGC	TCACTCATTAA	GGCAACCCAG	GCTTTACACT
6151	TTATGCTTCC	GGCTCGTATG	TTGTGTGGAA	TTGTGAGCGG	ATAACAAATT
6201	CACACAGGAA	ACAGCTATGA	CCATGATTAC	GAATTGGAGC	CGGGTACCG
6251	GOGATCCTCT	AGAGTGGACG	TGCAGGCGATG	CAAGCTTGGC	ACTGGCGTC
6301	GTTTTACAAC	GTOGTGACTG	GGAAAAACCT	GGCGTTAACCC	AACTTAATCG
6351	CCTTGCAGCA	CAATCCCTT	TOGCCAGCTG	GGGTAATAGC	GAAGAGGCGC
6401	GCACCGATCG	CGCTTCCCAA	CAGTTGGCGA	CGCTGAATGG	CGAATGGCGC
6451	TTTGGCTGGT	TTCGGCAOC	AGAAGGGGTG	CGCGAAAGCT	CGCTGGAGTG
6501	CGATCTTCT	GAGGCGATA	CGGTTGCGT	CGCGCTCAAAC	TGGCAGATGC

Figure 5

M13mp18 Nucleic Acid Sequence

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6551	AOGGTTACGA	TGOGOOCATC	TACACCAAOG	TAACCTATCC	CATTAOGGTC
6601	AATOOGOOGT	TTGTTCCAC	GGAGAAATCG	ACGGGTTGTT	ACTOGCTCAC
6651	ATTTAATGTT	GATGAAAGCT	GGCTACAGGA	AGGOCAGAOG	CGAATTATTT
6701	TTGATGGGCGT	TCCTATTGGT	TAAAAAAATGA	GCTGATTTAA	CAAAAATTAA
6751	AOGOGAATT	TAACAAAATA	TTAACGTTA	CAATTAAAT	ATTTGCTTAT
6801	ACAATCTCC	TGTTTTGGG	GCTTTCTGA	TTATCAACOG	GGGTACATAT
6851	GATTGACATG	CTAGTTTAC	GATTACOGTT	CATCGATTCT	CTTGTTGCT
6901	CCAGACTCTC	AGGCAATGAC	CTGATAGCT	TTGTAGATCT	CTCAAAAATA
6951	GCTACCCCTCT	COGGCATGAA	TTTATCAGCT	AGAAOGGTTG	AATATCATAT
7001	TGATGGTGAT	TTGACTGTCT	COGGCCTTC	TCACCCCTTT	GAATCTTAC
7051	CTACACATTA	CTCAGGCATT	GCATTTAAAA	TATATGAGGG	TTCTAAAAT
7101	TTTATCCCTT	GOGTTGAAAT	AAAGGCTTCT	COOGCAAAAG	TATTACAGGG
7151	TCATAATGTT	TTTGGTACAA	COGATTAGC	TTTATGCTCT	GAGGCCTTAT

Figure 5

M13mp18 Nucleic Acid Sequence

COMPLEMENTARY TO M₁₃

POSITION	5' * * * 3'	POSITION	
645	AGCAACACTATCATA	631	M ₁₃ /1
615	AOGAOGATAAAAACC	601	M ₁₃ /2
585	TTTGCAAAAGAAGT	571	M ₁₃ /3
555	AATAGTAAAATGTTT	541	M ₁₃ /4
525	CAATACTGOGGAATG	511	M ₁₃ /5
495	TGAATCCOCCTCAAA	481	M ₁₃ /6
465	AGAAAACGAGAATGA	451	M ₁₃ /7
435	CAGGTCTTACOCTG	421	M ₁₃ /8
405	AGGAAAGGGATTGC	391	M ₁₃ /9
375	AGGAAGGCGAAAGA	361	M ₁₃ /10

COMPLEMENTARY TO SS PHAGE DNA

POSITION	5' * * * 3'	POSITION	
351	ATATTTGAAGTCCTT	366	M ₁₃ /11
371	TCTTTTGATGCAAT	386	M ₁₃ /12
391	CTATAACTCAGGG	406	M ₁₃ /13
411	TGATTTATGGTCATT	426	M ₁₃ /14
431	GTTTAAAGCATTGTA	446	M ₁₃ /15
451	TATTTATGACGATTG	466	M ₁₃ /16
471	TATOCAGTCTAAACA	486	M ₁₃ /17
491	CTCTGGCAAAACTTC	506	M ₁₃ /18
511	TCGCTATTTGGTTT	526	M ₁₃ /19
531	AAAOGAGGGTTATGA	546	M ₁₃ /20

Figure 6

Primers for Nucleic Acid Production
Derived from M13mp18 Sequence

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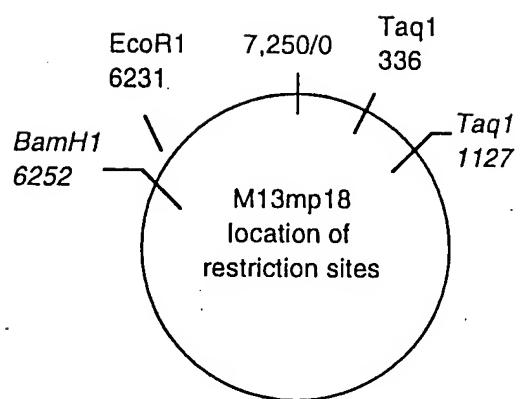
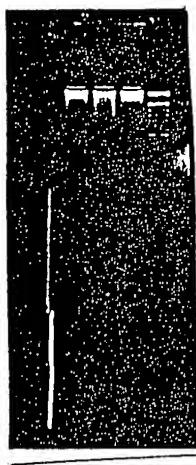


Figure 7

Appropriate M13mp18 Restriction Sites



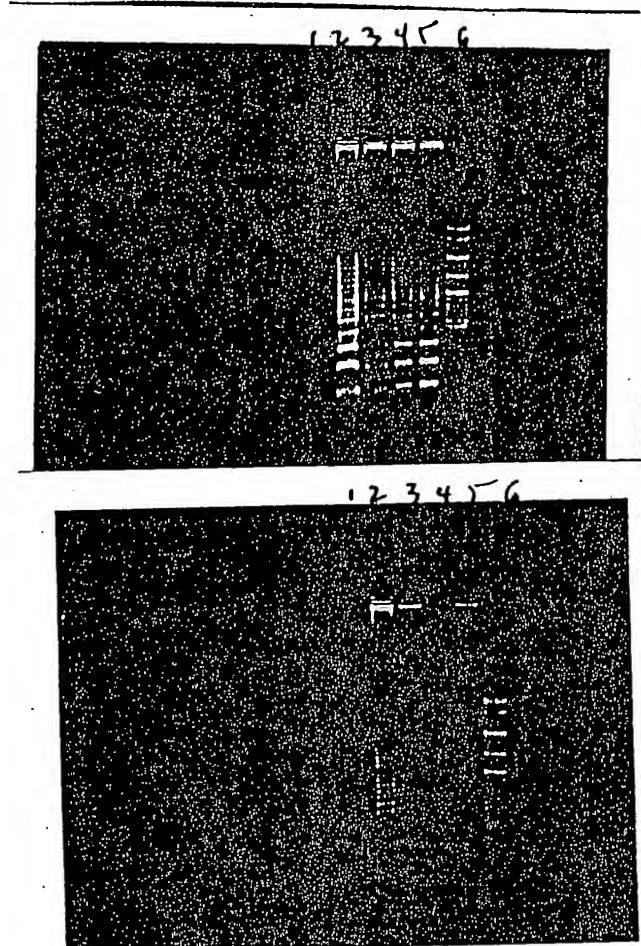
Lane 1: from calf thymus + Taq digested mp18 amplification reaction
Lane 2: from Taq digested mp18 amplification reaction
Lane 3: from calf thymus amplification reaction
Lane 4: ϕ X174 Hinf1 size marker

Figure 8



- Lane 1: no template
- Lane 2: mp18 template, phosphate buffer
- Lane 3: MspI/pBR322 size marker
- Lane 4: mp18 template, MOPS buffer

Figure 9



Top= (+) Template

Bottom= (-) Template

Lane 1: phosphate buffer

Lane 2: MES

Lane 3: MOPS

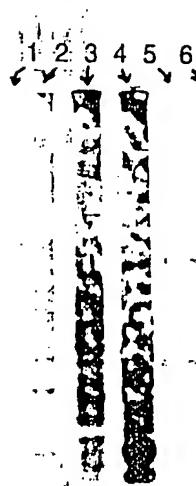
Lane 4: DMAB

Lane 5: DMG

Lane 6: pBR322/MspI size marker

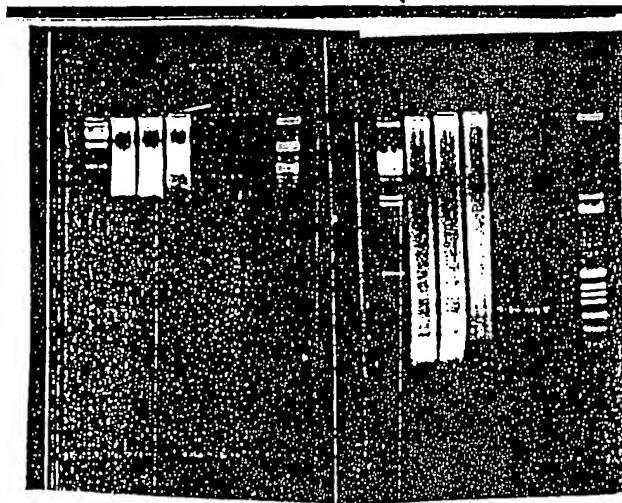
Figure 10

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- Lane 1: DMAB buffer, no template
- Lane 2: DMAB buffer, mp18 template
- Lane 3: DMG buffer, no template
- Lane 4: DMG buffer, mp18 template
- Lane 5: No reaction
- Lane 6: 200 ng Taq I digested mp18
size marker/positive control

Figure 11



First Time Interval Second Time Interval

Agarose Gel Analysis

- Lane 1: lambda Hind III marker
- Lane 2: Amp/Untreated
- Lane 3: Amp/Kinased
- Lane 4: Amp/Kinased/Ligated
- Lane 5: PCR/Untreated
- Lane 6: PCR/Kinased
- Lane 7: PCR/Kinased/Ligated
- Lane 8: ϕ X174/Hinf1 marker

Figure 12

18/23

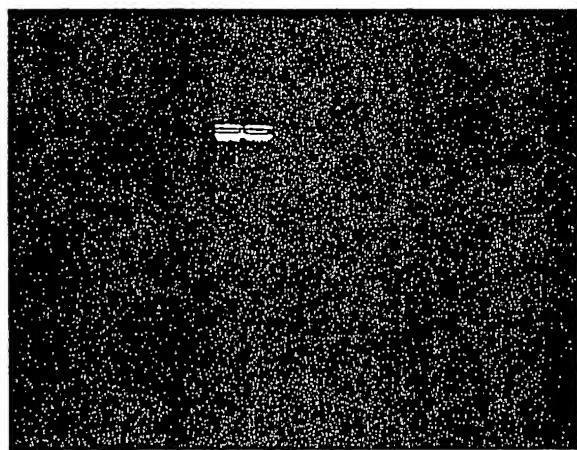
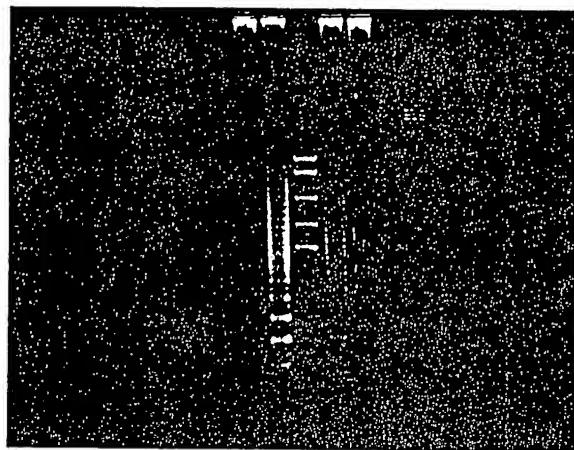


Figure 13

1 2 3 4 5 6



Lane 1: Primers alone
Lane 2: Primers + taq digested M13 DNA
Lane 3: Molecular weight markers
Lane 4: Primers + RNA
Lane 5: Primers alone
Lane 6: M13 digested DNA
Buffer was dimethyl amino glycine, pH 8.6

Figure 14

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Lane 1: Primers alone
Lane 2: Primers + taq digested M13 DNA
Lane 3: Molecular weight markers
Lane 4: Primers + RNA
Lane 5: Primers alone
Lane 6: M13 digested DNA
Buffer was dimethyl amino glycine, pH 8.6

Figure 15

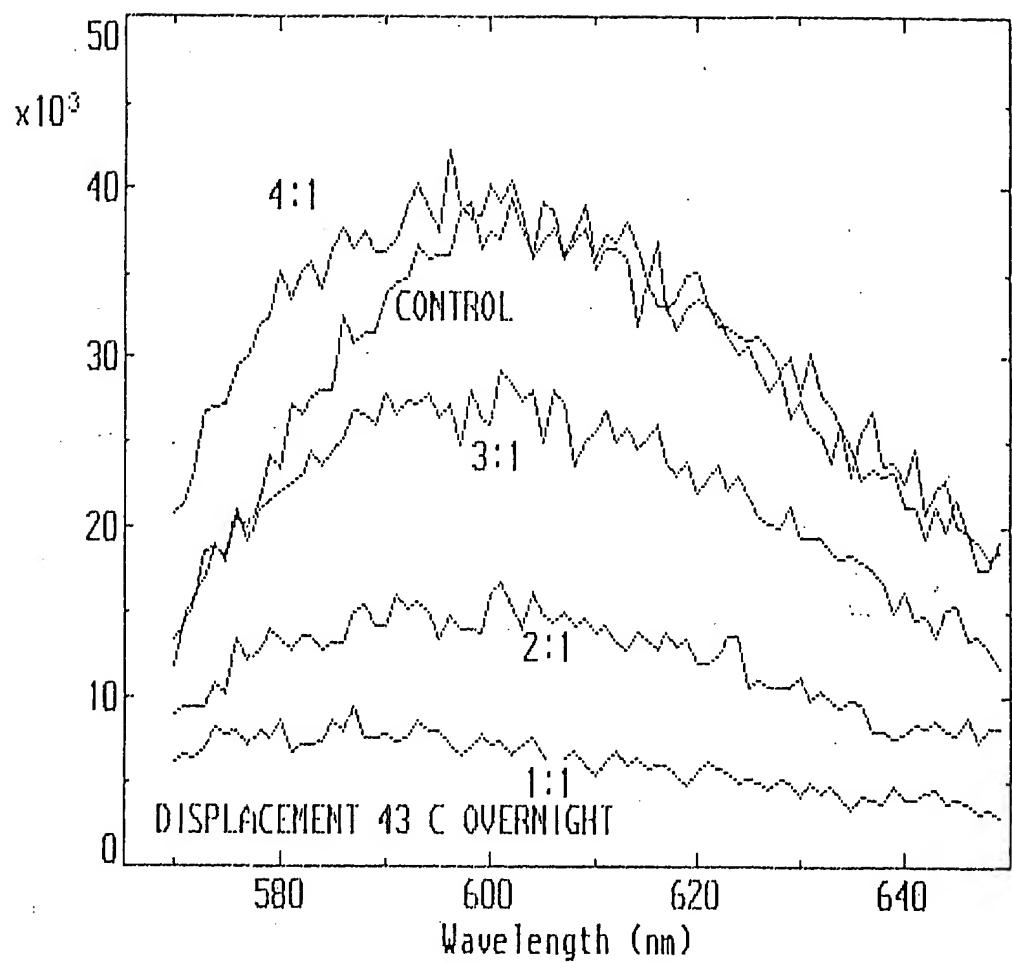


Figure 16

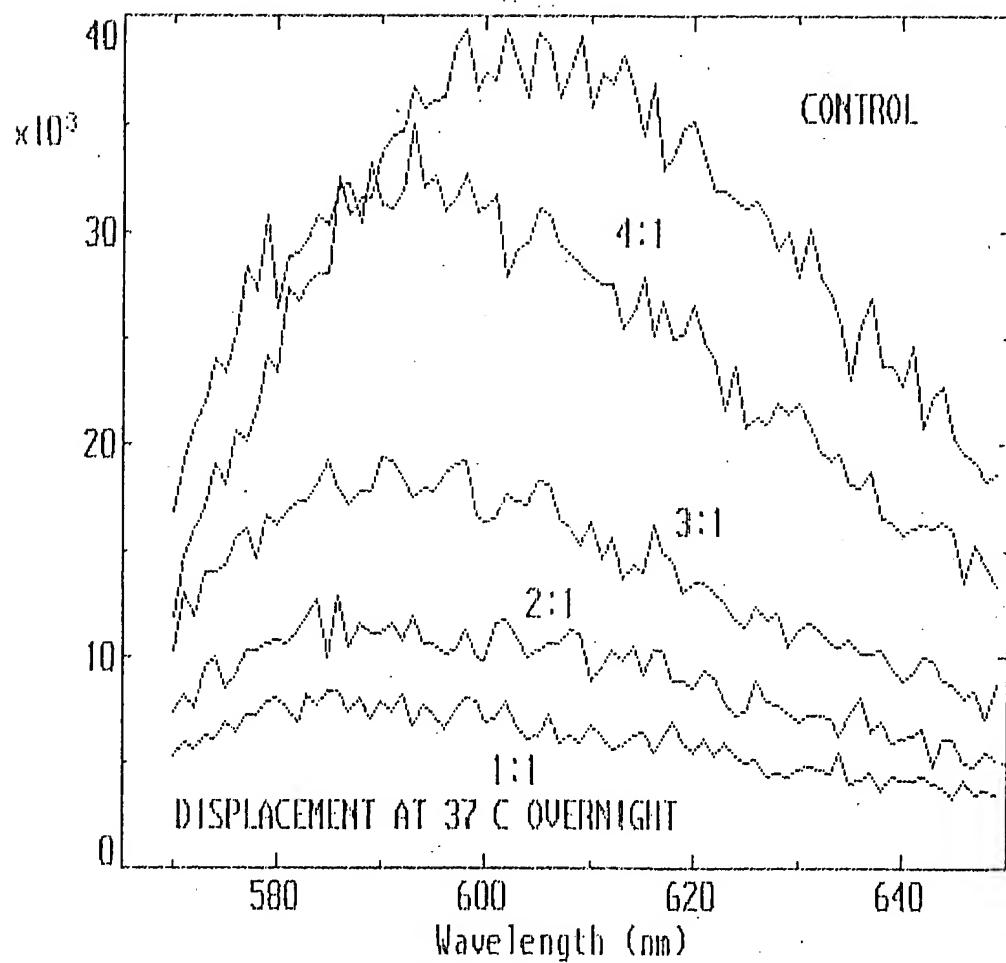


Figure 17

23/23

pIBI 31-BH5-2

fmct AUG of Lac z (T7 Promotor region....
LAC PROMOTOR..ATG ACC ATG ATT ACG CCA GAT ATC AAA TTA ATA CGA CTC ACT ATA
oligo 50-mer 3'- tac t'aa t'gc ggt* ct*a t'ag t'Vt aat* tat* gct* gag t'ga t'at* c-5'
10 base insert
T7 RNA Start {
T3 Promotor Region)
IGGG CTC ICCT TTA GTG ACG GTT AAT
..."} -- T3 Start Signal

pIBI 31 BSII/HCV

fmct AUG of Lac z (T3 Promotor region --) T3 RNA Start
LAC PROMOTOR ..ATG ACC ATG ATT ACG CCA AGC TCG AAA TTA ACC CTC ACT AAA /GGG
oligo 50-mer 3'- tac t'aa t'ac t'aa t'gc ggt* t'V--10 base insert--.....
(-- T7 Promotor Region)
MULTIPLE CLONING SITE + 390 BASE INSERT CTA /TAG TGA GTC CGT ATT AAT....
-- T7 Start Signal
5'-ct*a t'ag t'ga gt*c gt*a tt*a at".....

Figure 18